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RESEARCH HIGHLIGHT

Degeneration *Versus* Development: Hunting-Out the D-Unit of Huntington's Disease

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG trinucleotide repeat expansion exceeding a threshold length (> 35 repeats) in exon 1 of the huntingtin gene (*HTT*) [1]. The age at onset is typically 40–50 years, except for a very low percentage (approximately 6%) of juvenile-onset HD patients carrying 75 or more CAG repeats; longer CAG repeats predict an earlier onset [1]. The clinical features of HD vary among individuals but are typically characterized by progressive motor dysfunction, cognitive disorder, and psychiatric disturbance [1]. HD patients are usually healthy before the onset, showing no diagnosable symptoms [1]. Thus, HD is mainly considered to be an adult-onset neurodegenerative disorder with neurodegeneration and symptoms occurring only in middle-age for most patients. Meanwhile, the pathogenic mutant *HTT* gene as well as its mRNA and protein products are present in the patients' brains from the embryonic stage. It is intriguing whether these genetic products can cause imprinted changes in HD patients during development stages, leading to neurodegeneration and the disease symptoms occurring decades later. In a very recent study in *Science*, Barnat *et al*. studied HD from a novel angle to address this question and revealed novel potential pathogenic mechanisms [2].

Supported by well-established evidence, the cytotoxicity of mutant HTT protein (mHTT) has been considered the major contributor to HD pathogenesis, although the exact pathology remains unclear. mHTT may confer cytotoxicity by multiple co-existing mechanisms [1]. Different forms of mHTT disturb various cellular processes including gene transcription, gene translation, vesicular trafficking, cytoskeletal signaling, mitochondrial function, metabolism, synaptic plasticity, neural homeostasis, and protein clearance pathways [1, 3, 4]. Eventually, these dysregulated cell-autonomous processes accompanied by aberrant cell-cell interactions lead to neuronal death [1, 4]. Consistent with the gain-of-function pathogenic mechanism, mHTT rescues the embryonic lethality of partial wild-type HTT (wtHTT) inactivation in mice [5]. Also, hyper-expansion of polyglutamine (polyQ) leads to several other neurological disorders, all showing a prominent threshold effect [1]. The gain-of-function nature of mHTT justifies the therapeutic strategy to lower its level to treat HD. This strategy has been effective in many preclinical studies [6]. Interestingly, halting the expression of mHTT or lowering its level by genetic approaches can reverse the mHTT aggregate formation and progressive motor decline in mouse models even when the clinical symptoms have already been exhibited [7, 8]. However, these studies focused on lowering mHTT during adulthood and may have overlooked the developmental components of HD, probably because the mHTT gain-of-function is considered to be an adult-onset mechanism.

While most studies focus on the adult neurodegeneration, the developmental component of HD has been studied previously, but mainly from the perspective of the loss-of-function mechanism of wtHTT. The wtHTT is essential during development, as demonstrated by the embryonic lethality in *Hdh* (*HTT* homolog)-inactivated mice at embryonic day 7.5 [9]. The knockout embryos display defects in gastrulation, probably due to an altered nutritive function of the extra-embryonic tissues without HTT [10]. When embryonic lethality is bypassed through a low level

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of *Hdh* expression (< 50%), mice exhibit abnormal head morphology, malformed anatomical structure of the central nervous system, and wrongly-migrated neurons [5]. These anomalies indicate the critical function of wtHTT in early brain development. Electrophysiological assessments in HD mouse models have shown that changes in neuronal activity may occur during development and precede detectable behavioral symptoms [11]. However, the significance of the developmental component in typical HD patients is considered to be minor, because the majority of adult-onset HD patients with moderate CAG expansion (40–50 repeats) generally show no detectable symptoms before middle-age. In contrast, juvenile HD (jHD) patients become symptomatic before the age of 20, even as early as 2 years old. They typically show more severe symptoms like rigidity and a more rapid disease progression [1]. The disparity between these two types of HD patients underlies the theory that only mHTT with extreme pathological expansion is likely to affect neurodevelopment. Supporting this idea, previous studies have described reduced cranial volume, as well as altered neurogenesis and transcriptome, in HD models with extremely long CAG repeats (≥ 75) [12–14]. It is worth noting that most of these studies were performed using human iPSCs or HD mouse models; only a couple of studies describe the results with young HD gene carriers. Taken together, previous studies of HD-relevant developmental defects have focused on the loss-of-function of wtHTT or pathogenesis in jHD patients with extremely long CAG-repeats, with little relevance to the adult-onset mHTT cytotoxicity-induced neurodegeneration.

That is why the discovery by Barnat *et al*. seems striking: the developmental defects are present in human HD fetuses carrying merely 39–42 CAG repeats. This study provides unprecedented evidence that connects neurodevelopment to neurodegeneration in the common adult-onset HD. Barnat *et al*. examined defects in brain tissues from human fetuses carrying the HD-causing mutation with 39–42 CAG repeats. This tissue as well as HD mouse embryos showed marked developmental defects including mis-localization of mHTT in the ventricular zone, impairment in endosome activity, and disruption of neuroepithelial junctional complexes. As a result, the interkinetic migration process in progenitor cells is impaired, turning them from proliferation to differentiation towards the neuronal lineage [2]. As these progenitor cells are destined to become cortical neurons that project to the striatum, their pre-maturation will result in an imbalance in neurogenesis. This finding runs in parallel with the clinical features of the reduction of the striatum and the thinning of the cortex in HD patients. These results indicate that early brain development is disrupted in the human fetal cortex by mHTT with relatively small expansion.

The evidence above suggests that mHTT causes defects in human fetal brain development and challenges the view that the adult-onset HD is purely a neurodegenerative disorder. In our opinion, this has several important implications. First, it questions the rationale of mHTT-lowering therapeutic strategies in adult-onset HD patients after the onset. Therapeutic interventions may be needed early enough to ameliorate the dysregulated neurogenesis to assure normal development of the central nervous system before the circuitry is altered. Second, this study provides clues for neurodevelopmental studies. Further research is needed to explore the pathophysiological pathways underlying the deficits discovered, and mHTT may also interfere with other functions in neurogenesis. Other interesting directions that require further investigation include the compensatory mechanism *via* which the brain copes with these developmental abnormalities. This may explain why the symptoms are concealed before the age of onset and provide a mechanistic understanding of the progressive late-manifesting clinical features of HD.

While the study is exceptionally inspiring, additional studies are desired to further validate the findings with more thorough mechanistic details. One intriguing aspect is whether the developmental component is gain-of-function or loss-of-function. Previous studies mainly pointed towards a loss-of-function mechanism in the early development of HD, as heterozygous HTT-knockout mice or mice with partial HTT inactivation in the forebrain and testis show neuronal and behavioral phenotypes reminiscent of HD in the early stages [15, 16]. In the study by Barnat *et al*., it remains unclear whether the loss-of-function mechanism may also contribute to the abnormalities in HD human fetuses. The defects in transgenic mice carrying 111 CAG repeats are more likely to be gain-of-function, but the repeat length of this allele is much longer than typical HD patients, and the expression level of wtHTT *versus* mHTT was not tested. Another question is the potential role of expansion-harbored RNA. Previous studies demonstrated that RNA with trinucleotide or hexanucleotide repeats may potentially participate in the pathogenesis of several neurodegenerative diseases including HD and amyotrophic lateral sclerosis [17, 18]. The mouse model used by Barnat *et al*. lacks a control expressing mHTT with CAACAG repeats substituting CAG repeats to eliminate the potential role of RNA. Finally, the molecular mechanisms of the potential mHTT-induced developmental defects are lacking, and they are required to further validate the discovery and provide potential therapeutic entry points.

In conclusion, Barnat *et al*. present a very inspiring and significant discovery by demonstrating embryonic neurodevelopmental defects in HD patients with a CAG repeat number of approximately 40 (Fig. 1). This study implies

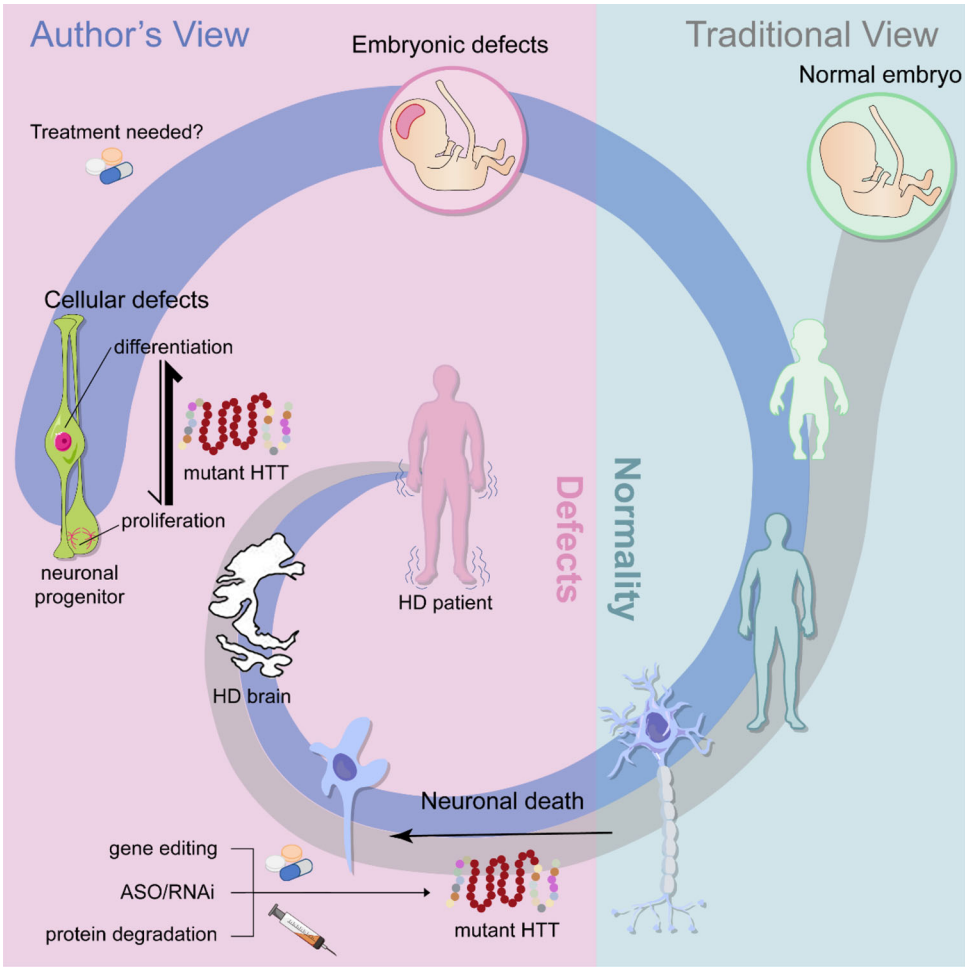

Fig. 1 Schematic for the possible pathogenic paradigm of Huntington's disease. The disease stage with defects and the normal stage are illustrated by a pink or cyan background, respectively. Traditional view (grey arrow flow): adult-onset HD patients (approximately 40 to 50 CAG) are normal and asymptomatic in early life, and mHTT may become detrimental decades after birth, causing neurodegeneration afterwards. This justifies the prevailing therapeutic strategy to lower mHTT level during adulthood after the onset. Author's view (blue arrow flow): mHTT may cause cellular defects in neuronal progenitor cells during embryonic development, leading to unbalanced excessive differentiation and subsequent abnormal neurodevelopment in the human fetus. This implies the necessity of lowering mHTT early during development before the alteration of brain circuitry.

potential defects "written" by mHTT at the embryonic stage, and these defects, while asymptomatic at early stages, may lead to the devastating disorder decades later. This challenges the prevailing strategy of lowering mHTT at the adult stage and suggests that early treatment at the developmental stage could be critical. It also provides intriguing directions for neurodevelopmental studies, as it would be fascinating to elucidate how the brain handles early developmental deficits so that typical HD patients remain healthy until middle age.

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